J Bone Miner Metab (2004) 22:236–240
DOI 10.1007/s00774-003-0473-z

Journal of
Bone and
Mineral
Metabolism

Impaired gamma carboxylation of osteocalcin in elderly women with type II diabetes mellitus: relationship between increase in undercarboxylated osteocalcin levels and low bone mineral density

Toshiyuki Horiuchi[1], Hirohito Kazama[1], Atsushi Araki[1], Junichiro Inoue[1], Takayuki Hosoi[1], Tsuneko Onouchi[2], Shouichi Mizuno[3], Hideki Ito[1], and Hajime Orimo[1]

[1] Department of Endocrinology, Tokyo Metropolitan Geriatric Medical Center, 35-2 Sakae-cho, Itabashi-ku, Tokyo 1173-0015, Japan
[2] Department of Clinical Research, Teikyo University School of Medicine, Chiba, Japan
[3] Department of Information and Simulation, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Abstract We conducted a cross-sectional examination of the role of serum vitamin K levels as they relate to bone metabolism in elderly women with type II diabetes mellitus (DM). Eighty-five elderly women with type II DM were enrolled. Three fractions of vitamin K, phylloquinone (PK), menaquinone 4 (menatetrenone; MK 4), and menaquinone 7 (MK 7), along with undercarboxylated osteocalcin (UcOC), intact osteocalcin (IOC), urinary deoxypyridinoline (udpd), urinary type I collagen N-telopeptide (NTx), and intact parathyroid hormone (IPTH) were measured. Bone mineral density was measured in the lumbar spine (LSBMD) by dual-energy X-ray absorptiometry (DXA), and T scores or Z scores were calculated. The patients were divided into two groups by T score, under −2.5 (osteoporotic group) and over −2.5 (non-osteoporotic group). UcOC levels in osteoporotics patients were significantly higher than those in the non-osteoporotic group (3.09 ± 3.94 vs 1.82 ± 1.76 ng/ml, $P = 0.02$). The correlation between Z score and logarithmic UcOC/IOC levels in type II DM showed a negative trend ($P = 0.07$) and a significantly and negatively association with logarithmic NTx ($r = -0.38$; $P = 0.001$). In osteoporotic DM, the UcOC/IOC ratio was significantly correlated with the Z score ($r = -0.61$; $P < 0.05$). Furthermore, logarithmic UcOC/IOC showed a negative correlation with logarithmic MK 7 ($r = -0.50$; $P = 0.001$). In conclusion, the reduction in LSBMD in elderly women with type II DM may be associated, in part, with a defect in γ-glutamylcarboxylation by vitamin K.

Key words type II DM · osteoporosis · vitamin K · undercarboxylated osteocalcin

Introduction

The mechanisms for the development of osteopenia or osteoporosis in type II diabetes mellitus (DM) remain controversial. There is conflicting evidence concerning bone loss in DM [1–5]. Similarly, DM is a risk factor for fractures [5–7], although there is no difference in the risk of fractures between type I DM and type II DM [1]. Patients with type I DM demonstrate a decrease in osteoblast function caused by insulin deficiency [8], whereas it is difficult to clarify the mechanism for the reduction in bone mineral density (BMD) in type II DM. Hyperglycemia causes increased fractional excretion of both Ca^{2+} and uric acid in type II DM patients [9]. Similarly, hyperglycemia influences the responses of osteoblasts to parathyroid hormone (PTH) and 1.25-dihydroxyvitamin D (1,25 $(OH)_2D_3$) [10,11]. However, many conflicting results have also been presented, in which hyperinsulinemia prevents bone loss in type II DM patients [12]. Recently, bone histomorphometry from the DM transiliac has demonstrated that the metabolic effects of poor glycemic control lead to low bone turnover, which retards age-related bone loss. A reduction in bone turnover may increase bone fragility, independent of bone density [13].

There is evidence that the levels of undercarboxylated osteocalcin (UcOC) increase in elderly people with bone fractures. A defect in vitamin K metabolism plays a role in hip fractures [14] and in BMD in elderly women [15]. Epidemiologically, a diet insufficient in vitamin K was also associated with higher morbidity, due to femoral neck fractures [16]. These studies endorse the view that vitamin K can prevent bone fractures in the elderly and is involved in bone metabolism.

The role of vitamin K in type II DM is not well known in terms of its effects on bone metabolism. In osteoporotics, vitamin K enhances the accumulation of osteocalcin in the extracellular matrix of human osteoblasts in vitro [17]. Whether or not there is a defect in vitamin K metabolism that causes osteoblast dysfunction in type II DM is still unclear. In order to elucidate the role of vitamin K in elderly diabetic patients with osteoporosis, we measured the levels of vitamin K, caliciotropic hormones, BMD, and bone biochemical

Offprint requests to: T. Horiuchi
(e-mail: thori@tmgh.metro.tokyo.jp)
Received: December 27, 2002 / Accepted: August 25, 2003

markers in elderly women with type II DM. We analyzed the significance of vitamin K metabolism from the standpoint of bone reduction in elderly women with type II diabetes.

Subjects and methods

Study design and subjects

Eighty-five subjects without vertebral deformities or fractures, as ascertained by lumbar X-ray, were selected to join this study. All subjects agreed to participate in the study by giving their informed consent under the guidelines of the Ethics Committee of the Tokyo Metropolitan Geriatric Medical Center. Patients taking prescribed bisphosphonates, alfacalcidol, menatrenone, ipriflavone, anabolic steroids, calcitonin, estrogen, insulin, thiazide, or statins were excluded by questionnaire, as were those frequently consumed "natto" (a type of fermented soybeans) or those who had consumed natto within a week priot to the study, and those who had symptoms of secondary osteoporosis caused by such conditions as hyperthyroidism, hyperparathyroidism, Cushing's syndrome, gastrectomy, or immobilization. The DM subjects were divided into two groups, by T scores (osteoporotics; $T < -2.5$) and non-osteoporotics ($T \geqq -2.5$). Parameters including glycohemoglobin, duration of DM, and years since menopause were compared between the two groups.

Bone biochemical markers and calciotropic hormone assays

Undercarboxylated osteocalcin (UcOC,), intact osteocalcin (IOC), and bone-specific alkaline phophatase (BAP) were chosen as markers of bone formation, while urinary deoxypyridinoline (udpd), and urinary type I collagen N-telopeptide (NTx) were used as markers of bone resorption. Blood and urine samples were collected in the morning following an overnight fast. Aliquots of blood and urine were frozen at −20°C until analyzed. Intact PTH (IPTH) and IOC were measured by immunoradiometric assays (IRMA). UcOC was measured with a specific enzyme-linked immunosorbent assay (ELISA) [18]. We also measured udpd and NTx by ELISA. Intraassay and interassay variances in the measurements were 6.9% and 3.5% respectively, for IPTH, 4.8% and 5.4% for IOC, 6.4% and 3.1% for NTx, 7.5% and 10.1% for udpd, and 7.2% and 7.9% for UcOC.

Vitamin K assay

Serum concentrations of three fractions of vitamin K, phylloquinone (PK), menaquinone 4 (menatetrenone; MK 4) and menaquinone 7 (MK 7), were determined by high-performance liquid chromatography separation with fluorescence detection [19]. Intraassay and interassay variances in the measurements were 3.8% and 3.0%, respectively, for PK, 3.8% and 4.4% for MK 4, and 5.5% and 2.7% for MK 7.

Bone mineral density (BMD) measurement

BMD was measured at the level of the second to fourth lumbar vertebrae (LSBMD) by dual-energy X-ray absorptiometry (DXA; Lunar IQ, Madison, WI, USA). T scores and Z scores for LSBMD were calculated as percentages of the values in young controls and in age-matched controls, respectively, based on the Lunar database of healthy Japanese women. Spillover in the calibration of the Lunar IQ apparatus was 7.8%.

Statistical analysis

All values are expressed as means ±SD unless otherwise indicated. Groups were compared using unpaired *t*-tests, with $P < 0.05$ considered statistically significant. The correlations between independent and dependent factors were analyzed by Pearson's coefficient, with SPSS software, after converting to logarithmic (log) values.

Results

MK 4 levels in DM was detectable in only 12 subjects in this study. Therefore, we did not include MK 4 in further analyses. Baseling age, body mass index (BMI), BMD, and years since menopause are shown in Table 1. Only UcOC levels were higher in osteoporotics than in non-osteoporotics (3.09 ± 3.94 vs 1.82 ± 1.76 ng/ml; $P = 0.02$), as shown in Table 2. No differences were observed between osteoporotics and non-osteoporotics in IPTH or bone biochemical markers (Table 2). There was a negative trend between Z score and log (UcOC/IOC) ($P = 0.07$), and a significant association with NTx in elderly DM patients ($P < 0.01$; Fig. 1a,b). The correlation between logarithmic UcOC/IOC and logarithmic MK 7 in elderly diabetics is shown in Fig. 2. Log (UcOC/IOC) was negatively and significantly correlated with log MK 7 ($r = -0.50$; $P = 0.001$). In osteoporotic DM patients, the ratio UcOC to IOC was significantly correlated with the Z score ($r = -0.61$; $P < 0.05$), as shown in Table 3 and in Fig. 3, but this correlation was not seen in non-osteoporotic DM patients.

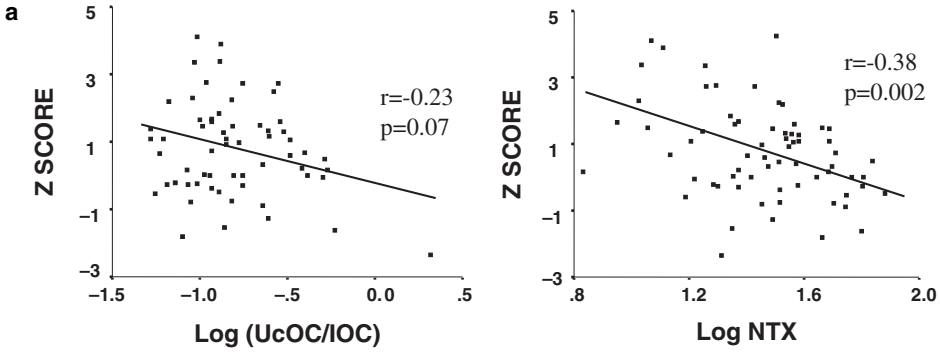

Fig. 1 **a** Correlation between Z score and log undercarboxylated osteocalcin (*UcOC*) in elderly diabetes mellitus (DM) patients. There is a negative trend between the Z score and log (UcOC/intact osteocalcin [*IOC*]) in ederly patients with DM **b** Correlation between Z score and log urinary type I collagen N-telopeptide (*NTx*) in DM. The Z score was significantly correlated with log NTx

Table 1. Comparison of baseline characteristics between elderly women with non-osteoporotic (non-op) DM and osteoporotic (op) DM

	Non-op (n = 70)	Op (n = 15)	P value
Age (years)	69.8 ± 3.8	71.7 ± 5.5	0.15
BMI	23.1 ± 2.7	21.3 ± 2.9	0.47
YSM	18.7 ± 3.5	21.2 ± 5.2	0.27
HbA1c (%)	7.30 ± 1.31	7.35 ± 1.14	0.96
Duration of DM (years)	13.1 ± 6.8	10.3 ± 8.9	0.65
L2-4 BMD	1.03 ± 0.13	0.732 ± 0.06	0.03
T score	−0.73 ± 1.14	−3.23 ± 0.55	0.02
Z score	1.16 ± 1.25	−0.8 ± 0.73	0.06

There were significant differences in L2-4 BMD values and T scores between the non-osteoporotic and osteoporotic DM patients
DM, diabetes mellitus; BMI, body mass index; YSM, years since menopause; Hb, hemoglobin; BMD, bone mineral density

Table 2. Comparisons of vitamin K, IPTH, and bone biochemical markers between non-osteoporotic DM and osteoporotic DM patients

	Non-op (n = 70)	Op (n = 15)	P value
PK (ng/ml)	1.1 ± 0.9	0.9 ± 0.5	0.22
MK7 (ng/ml)	8.1 ± 9.8	11.83 ± 13.5	0.27
IPTH (pg/ml)	31.1 ± 9.1	32.34 ± 12.7	0.38
UcOC (ng/ml)	1.82 ± 1.76	3.09 ± 3.94	0.02
IOC (ng/ml)	9.67 ± 3.37	9.85 ± 2.72	0.49
udpd (nmol/mmolCr)	6.3 ± 1.7	6.6 ± 1.7	0.81
NTx (nmolBCE/mmolCr)	32.1 ± 15.8	37.2 ± 15.8	0.77

UcOC levels in osteoporotic DM (n = 15) were significantly higher than those in non-osteoporotic DM (n = 70)
PK, phylloquinone; MK7 menaquinone 7; IPTH, intact parathyroid hormone; UcOC, urder-carboxylated osteocalcia; IOC, intact osteocalcin; udpd, urinary deoxypyridinoline; NTX, urinary type I collagen N-telopeptide

Table 3. Correlation coefficients between Z score and IPTH and bone biochemical markers in non-osteoporotic and osteoporotic DM patients

	Non-osteoporotics (n = 70)	Osteoporotics (n = 15)
IPTH	0.04	0.09
PK	0.01	−0.32
MK7	0.02	0.11
UcOC/IOC	−0.23	−0.61*
NTx	−0.12	0.20

* $P < 0.05$

Discussion

Among the baseline characteristics analyzed, there was a significant difference in UcOC values between non-osteoporotic DM and osteoporotic DM. However, neither PK nor MK 7 concentrations differed between non-osteoporotic and osteoporotic groups. MK 7 concentrations in osteoporotic DM women were slightly higher than those in non-osteoporotic group, although MK-7 levels were significantly correlated with UcOC/IOC in DM, as shown in Fig. 2. This paradox suggests that sufficient levels of vitamin K are present in DM, but that it does not promote the γ-glutamyl carboxylation of undercarboxylated osteocalcin (UcOC). Presumably, the γ-carboxylation of UcOC by vitamin K may be impaired in elderly patients with osteoporotic DM. It has been reported that anticoagulant therapy, which inhibits γ-glutamylcarboxylase activity, reduces bone mineral content at the lumbar spine and distal radius [20]. Hy-

perglycemia, as well as anticoaglants, may decrease γ-glutamylcarboxylase activity in diabetic osteoporotics. Thus, it is necessary to examine whether or not hyperglycemia results in a direct decrease in the activity of γ-glutamylcarboxylase. A decrease in γ-glutamylcarboxylase activity and an increase in undercarboxylated gla protein may induce the reduction of bone mineral content [21]. Our results are compatible with these results.

The onset of complications in DM, such as retinopathy and nephropathy, is dependent on the control of glucose and the duration of the disease. Bone loss is also associated with the duration of DM and insulin deficiency [22]. However, there were no significant correlations between the non-osteoporotic DM and osteoporotic DM patients in our study. Additionally, neither age nor BMI differed between the osteoporotics and non-osteoporotics. Hyperglycemia hampers the response of osteoblasts to $1,25(OH)_2D_3$ in vitro [23] and inhibits PTH secretion [23,24]. However, there was no significant difference between the non-osteoporotic DM and osteoporotic DM groups in glycohemoglobin, a parameter that reflects glucose control in diabetics. Glycohemoglobin levels in both groups were approximately 7.3%, indicating that glucose was moderately controlled. PTH secretion in elderly diabetics was also within the normal range in out assay. The induction of γ-glutamylcarboxylase as well as vitamin K epoxidase is regulated by $1,25(OH)_2D_3$ in vivo in rat kidney [25]. Similarly γ-glutamylcarboxylase in human osteoblasts is induced by pretreatment of $1,25(OH)_2D_3$ [26]. Therefore, it may be necessary to measure serum $1,25(OH)_2D_3$ concentrations in elderly diabetics with increased UcOC levels.

So far, NTx, among bone resorption markers, is the most sensitive predictor of bone loss in the lumbar spine. Markers of bone resorption can be used clinically to predict future BMD in postmenopausal women [27]. In our study, Z scores showed a significant association with NTx in DM patients. Similarly, UcOC/IOC showed a negative association with the Z score, which indicates that the increase of UcOC may be associated with an increase in bone resorption and a decrease of LSBMD in DM. It has been reported that vitamin K concentrations are not correlated with BMD, but may have an association with bone quality, as determined by ultrasonic transmitted velocity [28]. Thus, vitamin K seems to be more involved in bone quality than in bone quantity, as the carboxylation of osteocalcin as related to bone quality has been proposed as a possible mechanism for the prevention of bone fractures by vitamin K [29]. Bone volume and trabecular structure in rats subjected to longterm tail suspendsion are maintained by vitamin K_2 [30]. Diabetics, as well as astronauts, may rely on vitamin K metabolism for bone formation and bone resorption.

In summary, the levels of UcOC were higher in osteoporotic than in non-osteoporotic elderly patients with type II DM. MK 7 levels in osteoporotics were somewhat higher than those in non-osteoporotics, despite a significant negative association of MK 7 with UcOC concentrations. These results suggest that impaired vitamin K metabolism may be partly involved in

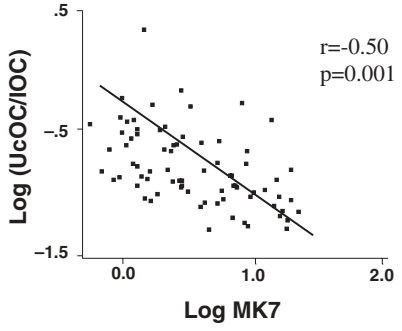

Fig. 2. Correlation between log UcOC/IOC and log menaquinone (*MK*) 7 in elderly DM women. Log (UcOC/IOC) was significantly correlated with log MK 7

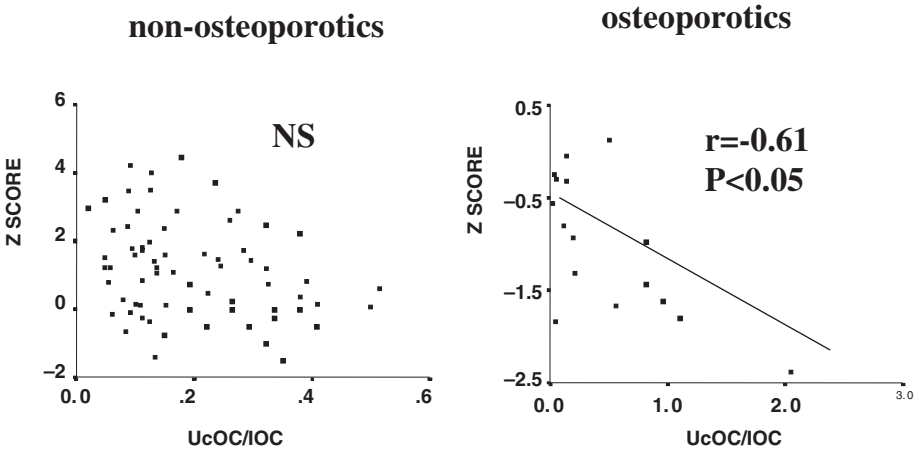

Fig. 3. Correlations between z score and UcOc/IOC in non-osteoporotic and osteoporotic DM patients. The Z score was significantly correlated with UcOC/IOC in osteoporotic DM patients. *NS*, not significant

the reduction in bone mineral content in elderly diabetic women.

References

1. Heath H, Melton LJ III, Chu CP (1980) Diabetes mellitus and risk of skeletal fracture. N Engl J Med 303:567–570
2. McNair P (1988) Bone mineral metabolism in human type I (insulin dependent) diabetes mellitus. Dan Med Bull 35:109–121
3. Selby PL (1988) Osteopenia and diabetes. Diabet Med 5:423–428
4. Melchior TM, Sorensen OH, Thamsborg G, Sylluluski R, Storm T (1990) Bone tissue alterations in diabetes. Front Diabetes 10:40–53
5. Bouillon R (1991) Diabetic bone disease. Calcif Tissue Int 49:155–160
6. Kelsey JL, Browner WS, Seeley DG, Nevitt MC, Cummings SR (1992) Risk factors for fractures of the distal forearm and proximal humerus. Am J Epidemiol 135:477–489
7. Meyer HE, Tverdal A, Falch JA (1993) Risk factors for hip fracture in middle aged Norwegian women and men. Am J Epidemiol 137:1203–1211
8. Rico H, Hernandez ER, Cabranes JA, Gomez-Castresana F (1989) Suggestion of a deficient osteoblast function in diabetes mellitus: The possible cause of osteopenia in diabetics. Calcif Tissue Int 45:71–73
9. Ishihara M, Shinoda T, Yamada T (1989) Co-occurrence of hypercalciuria and hypouricaemia in type 2 diabetic patients. Diabet Med 6:406–411
10. Yoshida O, Inaba M, Terada M, Shioi A, Nishizawa Y, Otani S, Morii H (1995) Impaired response of human osteosarcoma (MG-63) cells to human parathyroid hormone induced by sustain exposure to high glucose. Miner Electrolyte Metab 21:201–204
11. Inaba M, Nishizawa Y, Mita K, Kumeda Y, Emoto M, Kawagishi T, Ishimura E, Nakatsuka K, Shioi A, Morii H (1999) Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-dihydroxyvitamin D_3 in patients with type 2 diabetes. Osteoporosis Int 9:525–531
12. Meema HE, Meema S (1967) The relationship of diabetes mellitus and body weight to osteoporosis in elderly females. Can Med Assoc J 96:132–139
13. Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM (1995) Bone loss and bone turnover in diabetes. Diabetes 44:775–782
14. Szulk P, Chapuy MC, Meunier PJ, Delmas PD (1993) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 91:1769–1774
15. Szulk A, Arlot M, Chapuy MC, Duboeuf F, Meunier PJ, Delmas PD (1994) Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. J Bone Miner Res 9:1591–1595
16. Feskanich D, Weber P, Willet WC, Rockett H, Booth SL, Colditz GA (1999) Vitamin K intake and hip fractures in women: a prospective study. Am J Clin Nutr 69:74–79
17. Koshihara Y, Hoshi K (1997) Vitamin K2 enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro. J Bone Miner Res 12:431–438
18. Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delams P (1997) Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS study. J Clin Endocrinol Metab 82:719–724
19. Langenberg JP, Tjaden UR (1984) Determination of (endogenous) vitamin K1 in human plasma by reversed-phase high-performance liquid chromatography using fluorometric detection after post-column electrochemical detection. J of Chromatogr 305:61–72
20. Phillip WJ, Martin JC, Richardson JM, Reid DM, Webster J, Douglas AS (1995) Decreased axial and peripheral bone density in patients taking long-term warfarin. Q J Med 88:635–640
21. Fiore CE, Tamburino C, Foti R, Grimaldi D (1990) Reduced axial bone mineral content in patients taking oral anticoagulant. South Med J 83:538–542
22. Wakasugi M, Wakao R, Tawata M, Gan N, Koizumi K, Onaya T (1993) Bone mineral density measured by dual energy X-ray absorptiometry in patients with non-insulin-dependent diabetes mellitus. Bone 14:29–33
23. Inaba M, Terada M M, Koyama H, Yoshida O, Ishimura E, Kawagishi T, Okuno Y, Nishizawa Y, Otani S, Morii H (1995) Influence of high glucose on 1,25-dihydroxyvitain D3 induced effect on human osteoblast-like MG-63 cells. J Bone Miner Res 10:1050–1056
24. Fialip J, Moinade S, Thieblot P, Gaillard G, Cailleba A, Gentout C (1985) Calcium and phosphorus metabolism in insulin dependent and non-insulin dependent diabetics, well-controlled and poorly controlled. Diabet Metab 11:283–288
25. Karl PI, Carnes DL, Friedman PA (1985) Effect of 1,25-dihydroxychorecalciferol administration on the renal vitamin K-dependent carboxylating system. FEBS Lett 192:243–246
26. Miyake N, Hoshi K, Sano Y, Kikuchi K, Tadano K, Koshihara Y (2001) 1,25-dihydroxyvitamin D3 promotes vitamin K2 metabolism in human osteoblasts. Osteoporosis Int 12:680–687
27. Chaki O, Yoshikata I, Kikuchi R, Nakayama M, Uchiyama Y, Hirahara F, Gorai I (2000) The predictive value of biochemical markers of bone mineral density in postmenopausal Japanese women. J Bone Miner Res 15:1537–1544
28. Liu G, Peacock M (1998) Age-related changes in serum undercarboxylated osteocalcin and its relationship with bone density, bone quality, and hip fracture. Calcif Tissue Int 62:286–289
29. Sugiyama T, Kawai S (2001) Carboxylation of osteocalcin may be related to bone quality: a possible mechanism of bone fracture prevention by vitamin K. J Bone Miner Metab 19:146–149
30. Iwasaki Y, Yamato H, Murayama H, Sato M, Takahashi T, Ezawa I, Kurokawa K, Fukagawa M (2002) Maintenance of trabecular structure and bone volume by vitamin K2 in mature rats with long-term tail suspension. J Bone Miner Metab 20:216–222